Regulation of gluconate and ketogluconate production in Gluconobacter oxydans ATCC 621-H

Levering, P.R.; Weenk, G.; Olijve, W.; Dijkhuizen, L.; Harder, W.

Published in:
Archives of Microbiology

DOI:
10.1007/BF00446757

Publication date:
1988

Arch Microbiol (1988) 149:534–539

Archives of
Microbiology

Regulation of gluconate and ketogluconate production in *Gluconobacter oxydans* ATCC 621-H

P. R. Levering[1], G. Weenk[1,*], W. Olijve[1], L. Dijkhuizen[2], and W. Harder[2]

[1] Microbiological Research and Development Laboratories, Scientific Development Group, Organon Int., P. O. Box 20, NL-5340 BH Oss, The Netherlands

[2] Department of Microbiology, Biological Centre, University of Groningen, Kerklaan 30, NL-9751 NN Haren, The Netherlands

Abstract. *Gluconobacter* spp. possess the enzymic potential for two pathways of direct glucose oxidation. It has been proposed that the major part of glucose is oxidized to gluconate via NADP-dependent glucose dehydrogenase and that reoxidation of NADPH under these conditions proceeds via recycling of gluconate through ketogluconates. This hypothesis was tested in experiments in which *Gluconobacter oxydans* ATCC 621-H was grown in glucose-yeast extract medium containing [^{14}C]2-ketogluconate. As expected, glucose was almost quantitatively oxidized to gluconate, without further accumulation of 2- and 5-ketogluconate. Interestingly, the total amount of neither [^{14}C]2-ketogluconate nor [^{14}C]gluconate did change significantly during this oxidation phase, indicating that recycling of gluconate through ketogluconates did not occur. An analysis of enzyme activities in cell-free extracts of glucose-grown cells of *G. oxydans* ATCC 621-H showed that the membrane-bound glucose dehydrogenase was far more active than the NADP-linked glucose dehydrogenase. The activity of the latter enzyme constituted only 10–15% of that of quinoprotein glucose dehydrogenase and was far too low to match the in vivo rates of gluconate production in batch cultures of *G. oxydans*. It is concluded that under these conditions glucose is mainly oxidized to gluconate via the membrane-bound glucose dehydrogenase. Implications of these results for the regulation of ketogluconate formation are discussed.

Key words: *Gluconobacter oxydans* ATTC 621-H – Glucose oxidation – Gluconate formation – Ketogluconate formation – Regulation – [^{14}C]ketogluconate – Enzymology

The accumulation of gluconate and ketogluconates from glucose by *Gluconobacter* species has been subject of many studies. As a result, detailed information is currently available on cultivation conditions which favor their production. The underlying regulatory mechanisms that control the production of these compounds, however, are still poorly understood (Shchelkunova and Voinova 1969; Shinagawa et al. 1983; Stadler-Szöke et al. 1980; Stubbs et al. 1940; Weenk et al. 1984). Studies on the properties of a number of enzymes involved in the oxidation of glucose to ketogluconates (Ameyama and Adachi 1982a, b; Ameyama et al. 1981; Olijve 1978; Shinagawa et al. 1984) have revealed the enzymic potential for two pathways of direct glucose oxidation in *G. oxydans*, one proceeding extracellularly via membrane-bound dehydrogenases and another route involving soluble NADP-linked enzymes (Fig. 1).

During growth of *G. oxydans* ATTC 621-H and other *Gluconobacter* species on glucose in pH controlled batch cultures, a sequential accumulation of gluconate and ketogluconates is generally observed (Weenk et al. 1984). Initially, glucose is almost quantitatively converted into gluconate and only when the concentration of glucose has decreased below a certain value ketogluconate production starts (Weenk et al. 1984). This phenomenon is presumably not caused by repression of the relevant enzymes, since these are synthesized constitutively (De Ley and Dochy 1960; De Ley and Stouthamer 1959; Olijve 1978). Based on enzyme measurements in cell-free extracts and kinetics of glucose oxidation and glucose uptake by whole cells of *G. oxydans* ATCC 621-H, it has been suggested that glucose is mainly oxidized intracellularly to gluconate via the NADP-linked glucose dehydrogenase (Olijve 1978; Olijve and Kok 1979b). As a consequence, the rate of NADPH formation will be high. Since *G. oxydans* lacks a pyridine nucleotide transhydrogenase (Eagon 1963) and the observed NADPH oxidase activities were too low to account for complete oxidation of NADPH under these conditions, it has been proposed that NADP is regenerated via gluconate-recycling (Fig. 2). This model assumes that ketogluconates are produced by the membrane-bound gluconate dehydrogenase and reconverted into gluconate via the soluble ketogluconate reductases (Olijve 1978; Olijve and Kok 1979b). The in vivo operation of gluconate-recycling would also explain the absence of net ketogluconate accumulation during the first phase of glucose oxidation in batch cultures of *G. oxydans*. An analogous system has been suggested to operate in *Gluconobacter cerinus* during growth on fructose (Mowshowitz et al. 1974). An alternative mechanism for regeneration of NADP during glucose oxidation was postulated by Adachi et al. (1979). These authors provided evidence that the old yellow enzyme system (NADPH oxidase) in the presence of glucose-6-phosphate dehydrogenase and catalase, reoxidizes the NADPH produced during the conversion of glucose-6-phosphate to 6-phosphogluconate. Further evidence for the in vivo functioning of gluconate-recycling in *G. oxydans* was sought in experiments with ^{14}C-labelled 2-ketogluconate. The results of these experiments, and

* *Present address:* Nutricia Research, Nutricia Nederland b.v., P. O. Box 1, NL-2700 MA Zoetermeer, The Netherlands

Offprint requests to: P. R. Levering

Abbreviations: DCPIP, 2,6-dichlorophenolindophenol; PMS, phenazine methosulphate; PQQ, pyrrolo-quinoline quinone

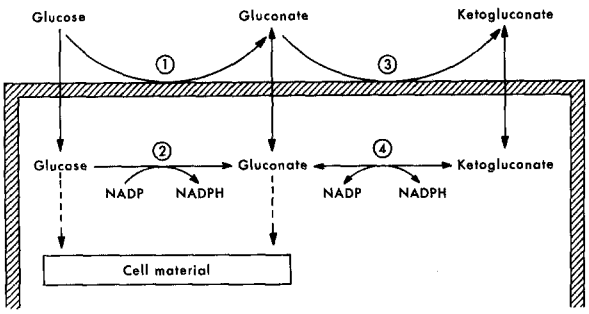

Fig. 1. Pathways of direct glucose oxidation in *Gluconobacter* species. The various reactions, indicated by numbers, are catalyzed by the following enzymes: *1* glucose dehydrogenase (membrane-bound) (EC 1.1.99.17); *2* glucose dehydrogenase (NADP) (EC 1.1.1.47); *3* gluconate dehydrogenase (membrane-bound) (EC 1.1.99.–); *4* ketogluconate reductase (NADP), 2-ketogluconate (EC 1.1.1–) or 5-ketogluconate (EC 1.1.1.69) as substrate

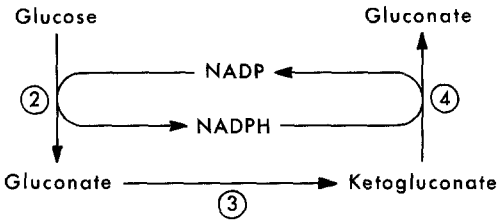

Fig. 2. Gluconate-recycling model. The numbers refer to enzymes listed in Fig. 1

additional enzymatic data, show that recycling of gluconate does not occur to a significant extent.

A preliminary report of this work has been presented elsewhere (Levering et al. 1987).

Materials and methods

Microorganisms

The *Gluconobacter* strains used in this study, *G. oxydans* subsp. *suboxydans* (ATCC 621-H) and *G. oxydans* subsp. *albidus* (IFO 3251 t_2), and their maintenance have been described previously (Weenk et al. 1984).

Media and cultivation

The organisms were either grown in a complex medium containing glucose and yeast extract at concentrations as indicated in the Results section or in a mineral salts medium, supplemented with vitamins and L-glutamine. This medium contained per litre of deionized water: $(NH_4)_2SO_4$, 2 g; $MgSO_4 \cdot 7\,H_2O$, 0.2 g; KH_2PO_4, 2.2 g; $Na_2HPO_4 \cdot 2\,H_2O$, 0.2 g; trace element solution according to Vishniac and Santer (1957), 0.4 ml. Additional iron ions were supplied as a 0.5% (w/v) $FeSO_4$/0.5% (w/v) nitrilotriacetic acid (NTA) solution, 1 ml l^{-1} and L-glutamine was added as the sole amino acid to a concentration of 200 mg l^{-1}. After heat sterilization of this medium the following filter-sterilized vitamins were added (per litre): calcium panthotenate, 0.5 mg; nicotinic acid, 0.4 mg; para-aminobenzoate, 0.4 mg. Glucose was heat-sterilized separately as a 40% (w/v) solution and used as carbon- and energy source at a final concentration of 100 mM.

Inocula for experiments in fermenters were prepared from frozen stocks (−70° C; 2 ml) in 250 ml shake flasks containing 50 ml of mannitol (1% w/v)/yeast extract (0.2% w/v) medium. Incubation was overnight on a rotary shaker at 28° C and 200 rpm.

Cultures to be used for analysis of enzymes were grown in Biostat E fermenters (B. Braun Melsungen AG, Melsungen, FRG) with a working volume of 5 l. The airflow rate was 60 l h^{-1} and the dissolved-oxygen tension, as recorded polarographically with a Clark-type electrode, was maintained above 25% air saturation by automatic adjustment of the stirrer speed (500–1,000 rpm). The temperature was kept at 28° C and the pH was controlled at 5.5 by automatic titration with 4 M NaOH or 10% (v/v) H_3PO_4.

Experiments with ^{14}C-labelled compounds were carried out in a magnetically-stirred 1-l fermenter (New Brunswick Scientific Co., Inc., Edison, NJ, USA) with a working volume of 0.5 l. The temperature of the cultures was 28° C and the pH was maintained at 5.5 by automatic titration with 2 M NaOH or 1 M HCl. Sufficient aeration was obtained by vigorous stirring (750 rpm) and a high airflow rate (30 l h^{-1}).

Growth of the cultures was monitored by measuring the absorbance at 650 nm using a Bausch and Lomb Spectronic 2000 spectrophotometer (Bausch and Lomb Inc. Rochester, NY, USA). For the assay of enzyme activities cells were harvested by centrifugation at 10,000 × *g* for 10 min at 4° C, washed twice with 50 mM potassium phosphate buffer pH 6.0 containing 5 mM $MgCl_2$ and resuspended in this buffer to a final concentration of 20–40 mg dry weight ml^{-1}. These suspensions were used immediately for enzyme assays or stored at −70° C until required. When used within 1 month, extracts of frozen cells exhibited enzyme activities of more that 90% of those obtained with freshly harvested cells.

Preparation of cell-free extracts and enzyme assays

Suspensions were sonicated with a Branson Sonifier B-12 Cell Disruptor (Branson Sonic Power Co., Danbury, CT, USA) operating at 45 W output at 0° C for 15 × 15 s, with intermitting cooling periods of 30 s. Whole cells were removed by centrifugation at 10,000 × *g* for 5 min at 4° C and the supernatant, which contained 7–16 mg protein ml^{-1}, was used as the cell-free extract for the determination of enzyme activities. All assays were performed at 28° C with a Bausch and Lomb Spectronic 2000 spectrophotometer (Bausch and Lomb Inc., Rochester, NY, USA). The observed rate was linear for at least 2 min and was proportional to the amount of extract added. Enzyme activities are expressed as units mg^{-1} protein. One unit of activity is defined as that amount of enzyme catalyzing the transformation of 1 µmol of substrate in 1 min. The molar extinction coefficients (M^{-1} cm^{-1}) of NADPH at 340 nm and of 2,6-dichlorophenolindophenol at 600 nm and pH 5.5 were 6.22×10^3 and 7.0×10^3, respectively. In the assays of the NADP-dependent dehydrogenase and reductases Triton X-100 was added to inhibit the high spontaneous rate of NADPH oxidation as was reported for rat liver microsomes (Boutin 1986). In this respect Triton X-100 turned out to be a more efficient inhibitor than potassium cyanide or sodium azide.

Enzyme assays were performed as described previously (Olijve 1978), except that the membrane-bound glucose dehydrogenase (EC 1.1.99.17) was not measured polarographically, but with a spectrophotometric assay employing PMS/DCPIP as electron-acceptors (Ameyama et al. 1981).

Glucose dehydrogenase (EC 1.1.99.17). The reaction mixture (1 ml) contained: potassium phosphate buffer pH 5.5, 75 µmol; 2,6-dichlorophenolindophenol, 0.15 µmol; phenazine methosulphate, 2 µmol; and extract. The reaction was started by the addition of 20 µmol glucose.

Glucose dehydrogenase (NADP-dependent) (EC 1.1.1.47). The reaction mixture (1 ml) contained: potassium phosphate buffer pH 7.4, 75 µmol; NADP, 0.5 µmol; Triton X-100, 0.2% (v/v); and extract. The reaction was started by the addition of 60 µmol glucose.

Gluconate dehydrogenase (EC 1.1.99.–). The reaction mixture (1 ml) was the same as for the particulate glucose dehydrogenase (EC 1.1.99.17), except that 20 µmol potassium gluconate was used as the substrate.

5-Ketogluconate reductase (NADP-dependent) (EC 1.1.1.69). The reaction mixture (1 ml) contained: potassium phosphate buffer pH 7.4, 75 µmol; NADPH, 0.15 µmol; Triton X-100, 0.2% (v/v); and extract. The reaction was started by the addition of 2.5 µmol potassium 5-ketogluconate.

2-Ketogluconate reductase (NADP-dependent) (EC 1.1.1.–). The reaction mixture (1 ml) was the same as for 5-ketogluconate reductase, except that 10 µmol sodium 2-ketogluconate was used as the substrate.

Preparation of [^{14}C]2-ketogluconate

Since ^{14}C-labelled ketogluconates are not commercially available, it was decided to prepare [^{14}C]2-ketogluconate fermentatively from [U-^{14}C]glucose by a culture of *G. oxydans* subsp. *albidus* (IFO 3251 t_2), as described above. The total amount of radioactivity of the 2-ketogluconate preparation obtained in this way was determined by liquid scintillation counting. Samples (10 or 20 µl) were diluted in 2 ml Pico-fluor 30 scintillation fluid and measured in a Packard Tri-Carb model 2000 CA analyzer (Packard Instrument Co., Downers Grove, IL, USA). Counting efficiences were calculated from internal quenching calibration curves.

Protein determinations

Protein was determined by the method of Lowry et al. (1951), using bovine serum albumin as a standard.

Substrate/product analyses

Samples from cultures for the determination of substrates and products were obtained by centrifugation and subsequent filtration through a 0.2 µm filter (Sartorius GmbH, Göttingen, FRG).

Glucose was assayed enzymatically (GOD-Perid method of Boehringer Mannheim GmbH, FRG).

Gluconate was either determined with gluconate kinase/6-phosphogluconate dehydrogenase (Boehringer Mannheim GmbH, FRG) or by a HPLC method, which was also used for the analysis of 2- and 5-ketogluconate (see below). Prior to the enzymatic analysis the samples were diluted 10-fold in 0.1 M K_2HPO_4 and incubated overnight, in order to ensure complete hydrolysis of glucono-δ-lactone (the primary product of glucose oxidation) to gluconate.

Gluconate and 2- and 5-ketogluconate were detected by High-Performance Liquid Chromatography using a Bio-Sil TSK DEAE-2 SW column (250 × 4.5 mm) (Bio-Rad Laboratories, Richmond, CA, USA). Sample volumes of 10 µl were injected. The temperature during the chromatography was kept constant at 40°C. Each run was carried out isocratically using 60 mM KH_2PO_4 elution buffer, adjusted to pH 3.75 with phosphoric acid. Complete hydrolysis of glucono-δ-lactone was achieved by adding 0.05 ml 1 M K_2HPO_4 to 1 ml of sample. After overnight incubation these solutions were diluted with a minimum of 0.95 ml 50 mM KH_2PO_4 (adjusted to pH 3.9 with phosphoric acid). Chromatography was performed with a flow rate of 0.5 ml min^{-1} and gluconate, 5-ketogluconate and 2-ketogluconate peaks were detected by UV absorbance at 210 nm after approx. 13 min, 15.5 min and 17 min, respectively. The detection limit was 1 mM. Radioactivity ^{14}C present in these peaks was measured continuously by using a Ramona-D detector equipped with an internal CaF scintillator (400 µl volume) (Isomess GmbH, Straubenhardt, FRG), in series with the UV-detector.

Chemicals

[U-^{14}C]glucose was obtained from NEN Chemicals (Boston, MA, USA) as dry powder. All biochemicals used in enzyme assays were from Sigma Chemical Co. (St. Louis, MO, USA), except for NADP and NADPH, which were purchased from Boehringer Mannheim GmbH (FRG). Pico-fluor 30 was obtained from Packard Instrument Co. (Downers Grove, IL, USA).

Results

Production of [^{14}C]2-ketogluconate from [^{14}C]glucose by Gluconobacter oxydans subsp. albidus (IFO 3251 t_2)

Radioactive ketogluconate was prepared by using *G. oxydans* subsp. *albidus* (IFO 3251 t_2), which was selected for its high selectivity and rate of glucose conversion into 2-ketogluconate (Weenk et al. 1984). Therefore, this organism was grown in a yeast extract (0.5% w/v) medium supplemented with 1 mCi (3.7×10^7 Bq) [U-^{14}C]glucose (final concentration 200 mM). After a total incubation time of two days the culture was harvested by centrifugation and the supernatant was filter-sterilized and stored at −20°C until required. This solution (475 ml) contained in total 2.8×10^7 Bq. Glucose and 5-ketogluconate concentrations were below the detection limits, whereas the final concentrations of gluconate and 2-ketogluconate were 8.2 and 168 mM, respectively.

Fate of [^{14}C]2-ketogluconate during the oxidation of excess glucose in batch cultures of Gluconobacter oxydans ATCC 621-H

The availability of radioactive ketogluconate allowed us to test the hypothesis that gluconate is being recycled during

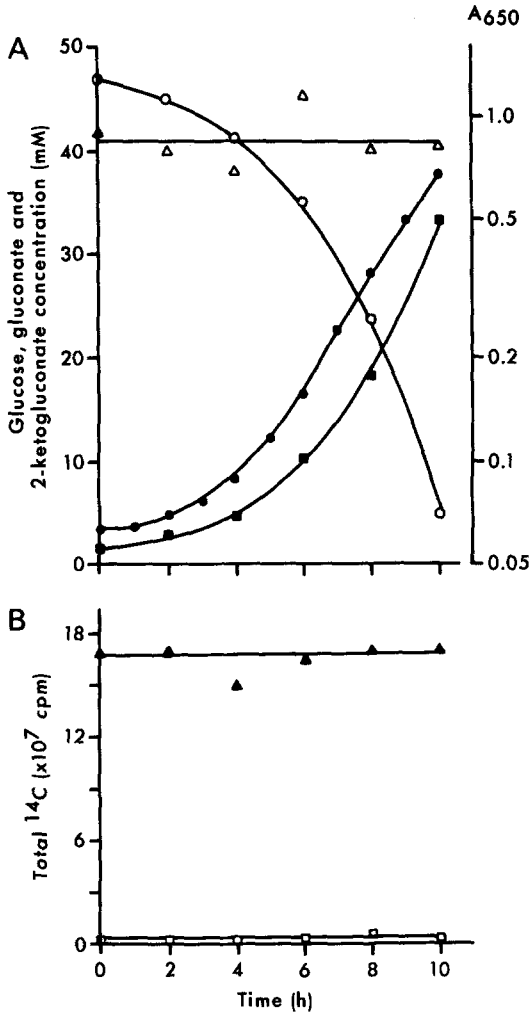

Fig. 3A, B. Growth of *Gluconobacter oxydans* ATCC 621-H in batch culture in a glucose (50 mM), yeast extract (0.5%, w/v) medium, supplemented with [^{14}C]2-ketogluconate (40 mM; approx. 0.4 MBq/mmol). **A** ●, absorbance; ○, glucose concentration; ■, gluconate concentration; △, 2-ketogluconate concentration. **B** □, total amount of [^{14}C]gluconate; ▲, total amount of [^{14}C]2-ketogluconate

exponential growth of *G. oxydans* on glucose (Fig. 2). The operation of such a cycle in vivo should result in dilution of radioactivity present in ketogluconates and appearance of radioactivity in the gluconate pool. Therefore, *G. oxydans* ATCC 621-H was grown in yeast extract medium containing 50 mM glucose, supplemented with part of the [^{14}C]2-ketogluconate solution, prepared as described above. As can be seen from Fig. 3A, the growth rate of the organism steadily increased from $t = 0$ h onwards, to reach a phase of exponential growth with a doubling time of 1.8 h, as normally observed during growth on glucose alone in complex media (Olijve and Kok 1979a; Weenk et al. 1984). The metabolite concentrations also exhibited a normal pattern, i.e. a near-quantitative oxidation of glucose to gluconate and an apparent absence of 2- and 5-ketogluconate formation (Fig. 3A). Most importantly, an analysis of the distribution of radioactivity in the various pools of metabolites showed that the total amount of neither [^{14}C]2-ketogluconate nor [^{14}C]gluconate in the culture did alter significantly during the oxidation of glucose (Fig. 3B).

Table 1. Enzyme activities in cell-free extracts of *G. oxydans* (ATCC 621-H) during the early- to mid- exponential growth phase on glucose (100 mM) in either a complex [1% (w/v) yeast extract] or a mineral salts medium (activities are expressed as units mg^{-1} of protein)

Enzyme	Medium	
	Complex	Mineral salts
Glucose dehydrogenase (particulate)	2.9 – 3.9	2.5 – 2.8
Glucose dehydrogenase (NADP)	0.38 – 0.42	0.15 – 0.32
Gluconate dehydrogenase (particulate)	0.03 – 0.05	0.04 – 0.08
2-Ketogluconate reductase (NADPH)	0.07 – 0.08	n.d.
5-Ketogluconate reductase (NADPH)	0.01 – 0.02	n.d.

n.d. not determined
Data obtained in various independent experiments

This indicates that recycling of gluconate through ketogluconates did not take place.

Enzymology of glucose utilization by Gluconobacter oxydans ATCC 621-H

The gluconate-recycling model was originally put forward following the observation that NADP-dependent glucose dehydrogenase is the more active enzyme in the first phase of glucose utilization in batch culture. The results obtained in the experiment described above prompted us to critically reexamine the role of the various enzymes involved in the direct pathway of glucose oxidation (Fig. 1). The organism was grown in either a yeast extract or a mineral salts medium containing 100 mM glucose and cells were harvested when in the early – to mid – exponential growth phase. Although a considerable variation in activities between different cell samples was observed, it is clear that the membrane-bound glucose dehydrogenase is far more active than the soluble NADP-linked dehydrogenase, both in cells grown in complex and mineral salts media (Table 1). The activity of the latter enzyme was only 10 – 15% of that of the particulate glucose dehydrogenase and far too low to account for the in vivo rates of glucose oxidation and gluconate production in batch cultures of *G. oxydans* growing in the presence of excess glucose (approximately 2.5 – 4.0 μmol min^{-1} mg^{-1} protein). Under these conditions the major part of the glucose thus may be oxidized to gluconate via the membrane-bound glucose dehydrogenase. An analysis of activities of other enzymes involved in the direct oxidation of glucose, namely particulate gluconate dehydrogenase and the ketogluconate reductases (see Fig. 2), demonstrated relatively low yet significant levels of these enzymes (Table 1).

Discussion

The ability of *Gluconobacter* to produce gluconate from glucose has been recognized for a long time (Boutroux 1886). Over the years it has become clear that this is not a property

peculiar for this genus, but that several strains of *Pseudomonas* (Lessie and Phibbs 1984), *Escherichia coli* (Hommes et al. 1984), *Klebsiella aerogenes* (Neijssel et al. 1983), *Acinetobacter* spp. (van Schie et al. 1984, 1987), *Agrobacterium* spp. (Linton et al. 1987; van Schie et al. 1987), *Rhizobium* spp. (van Schie et al. 1987) and *Aspergillus niger* (Meiberg and Spa 1983) also possess this ability. With the exception of the latter organism, which possesses glucose oxidase (EC 1.1.3.4), they all employ a membrane-bound pyridine nucleotide-independent glucose dehydrogenase (EC 1.1.99.17) to catalyze the oxidation of glucose to gluconate. This enzyme belongs to the class of quinoproteins, which possess pyrrolo-quinoline quinone (PQQ) as the prosthetic group (Duine and Frank 1981). Unlike the other organisms mentioned above, *Gluconobacter* species also express, in addition to a membrane-bound activity, a soluble NADP-linked glucose dehydrogenase (EC 1.1.1.47) (Fig. 1). Early on it was suggested that the latter enzyme plays the most important role in glucose oxidation in *G. oxydans* (Olijve 1978; Olijve and Kok 1979b). Since this would result in a high rate of NADPH formation from glucose oxidation, the "gluconate-recycling" model was proposed as a mechanism for regeneration of NADP (Fig. 2). The present study aimed to further test the in vivo operation of this cycle. Incubation of *G. oxydans* in medium with glucose and [^{14}C]2-ketogluconate, however, did not result in dilution of label in 2-ketogluconate and/or appearance of label in gluconate (Fig. 3).

We subsequently decided to undertake a detailed enzymatic analysis of glucose-grown cells of *G. oxydans* ATCC 621-H (Table 1) and found that the activities of the membrane-bound glucose dehydrogenase were much higher than those reported previously (Olijve 1978; Olijve and Kok 1979b). This discrepancy is readily traced back to the use of different assay methods of this enzyme. Whereas the latter authors employed a polarographic assay, we used a spectrophotometric method with PMS and DCPIP as artificial electron acceptors (Ameyama et al. 1981). The activities of quinoprotein glucose dehydrogenase observed in the present study (Table 1) are in close agreement with the in vivo glucose oxidation rates in batch cultures of *G. oxydans*. It thus appears far more likely that this enzyme plays a key role in glucose metabolism by this organism. Definite proof, however, should be obtained in enzyme inhibition studies and analyses of appropriate mutants.

The results of the experiment with [^{14}C]2-ketogluconate (Fig. 3) also necessitate a revision of the views about the regulatory mechanisms underlying the sequential production of gluconate and ketogluconates from glucose. There is some circumstantial evidence to indicate that the actual rate of glucose oxidation is a major factor in controlling the accumulation of ketogluconates. Thus, Weenk et al. (1984) reported that the onset of ketogluconate formation during growth on glucose in batch cultures coincided with a decrease of the specific glucose oxidation rates of the cultures. Simultaneous formation of gluconate and ketogluconate was also observed, when *G. oxydans* ATCC 621-H was grown in glucose-limited chemostat cultures and the ratio of these products was found to be dependent on the dilution rate employed (Olijve and Kok 1979b). In general, membrane-bound dehydrogenases donate the electrons, which derive from the oxidation of their substrates, directly to components of the electron transport chain. In case of flavoproteins, such as gluconate dehydrogenase (EC 1.1.99.3), these components usually are ubiquinone or cytochrome b (Matsushita et al. 1979). There is some evidence to indicate that PQQ-glucose dehydrogenases are also linked to the respiratory chain at the level of ubiquinone or cytochrome b (Beardmore-Gray and Anthony 1986; Duine et al. 1984; Matsushita et al. 1980, 1982; van Schie et al. 1985). With respect to *G. oxydans*, one might speculate that, during the first phase of growth on glucose in batch cultures (excess glucose), the oxidation of glucose mediated by the particulate glucose dehydrogenase proceeds so rapidly that the respiratory chain becomes saturated with electrons. Consequently, the redox components will be unable to accept electrons from the gluconate dehydrogenase, resulting in the observed failure of the cells to oxidize gluconate under these conditions. Such a regulatory model of competition of various respiratory chain components for electrons will require further investigation. Attractive options to do so include the use of membrane vesicles and, furthermore, the use of appropriate mutants.

Acknowledgements. The investigations were partly supported by the Foundation for Fundamental Biological Research (BION), which is subsidized by the Netherlands Organization for the Advancement of Pure Research (ZWO). Thanks are due to Mrs. Anjo van Heijst and Mr. C. Lucas for their skilful technical assistance and the help of Mrs. Martha Krancher and Mrs. Rina van de Leemput in the preparation of the manuscript is gratefully acknowledged.

References

Adachi O, Matsushita K, Shinagawa E, Ameyama M (1979) Occurrence of old yellow enzyme in *Gluconobacter suboxydans*, and the cyclic regeneration of NADP. J Biochem 86:699–709

Ameyama M, Adachi O (1982a) 5-Keto-D-gluconate reductase from *Gluconobacter suboxydans*. Meth Enzymol 89:198–202

Ameyama M, Adachi O (1982b) 2-Keto-D-gluconate reductase from acetic acid bacteria. Meth Enzymol 89:203–210

Ameyama M, Shinagawa E, Matsushita K, Adachi O (1981) D-Glucose dehydrogenase of *Gluconobacter suboxydans*: solubilization, purification and characterization. Agric Biol Chem 45:851–861

Beardmore-Gray M, Anthony C (1986) The oxidation of glucose by *Acinetobacter calcoaceticus*: interaction of the quinoprotein glucose dehydrogenase with the electron transport chain. J Gen Microbiol 132:1257–1268

Boutin JA (1986) Inhibition of microsomal NAD(P)H oxidation by Triton X-100. J Biochem Biophys Meth 13:171–178

Boutroux L (1886) Sur une fermentation acide du glucose. Compt Rend 102:924–927

Duine JA, Frank Jzn J (1981) Quinoproteins: a novel class of dehydrogenases. Trends Biochem Sci 6:278–280

Duine JA, Frank Jzn J, Jongejan JA, Dijkstra M (1984) Enzymology of the bacterial methanol oxidation step. In: Crawford RL, Hanson RS (eds) Microbial growth on C_1 compounds. Am Soc Microbiol, Washington DC, pp 91–96

Eagon RG (1963) Rate limiting effects of pyridine nucleotides on carbohydrate catabolic pathways of microorganisms. Biochem Biophys Res Comm 12:274–279

Hommes RWJ, Postma PW, Neijssel OM, Tempest DW, Dokter P, Duine JA (1984) Evidence of quinoprotein glucose dehydrogenase apoenzyme in several strains of *Escherichia coli*. FEMS Microbiol Lett 24:329–333

Lessie TG, Phibbs PV (1984) Alternative pathways of carbohydrate utilization in pseudomonads. Annu Rev Microbiol 38:359–387

Levering PR, Lucas C, van Heijst A, Olijve W (1987) Regulation of ketogluconate formation by *Gluconobacter* species. Abstr 4th Eur Congress on Biotechnology, Amsterdam, The Netherlands

Ley J de, Dochy R (1960) On the localization of oxidase systems in *Acetobacter* cells. Biochim Biophys Acta 40:277–289

Ley J de, Stouthamer AJ (1959) The mechanism and localization of hexonate metabolism in *Acetobacter suboxydans* and *Acetobacter melanogenum*. Biochim Biophys Acta 34:171–183

Linton JD, Woodard S, Gouldney DG (1987) The consequence of stimulating glucose dehydrogenase activity by the addition of PQQ on metabolite production by *Agrobacterium radiobacter* NCIB 11883. Appl Microbiol Biotechnol 25:357–361

Lowry OH, Rosebrough HJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275

Matsushita K, Shinagawa E, Adachi O, Ameyama M (1979) Membrane-bound D-glucose dehydrogenase from *Pseudomonas aeruginosa*. Its kinetic properties and a reconstitution of gluconate oxidase. J Biochem 86:249–256

Matsushita K, Ohno Y, Shinagawa E, Adachi O, Ameyama M (1980) Membrane-bound D-glucose dehydrogenase from *Pseudomonas* sp: solubilization, purification and characterization. Agric Biol Chem 44:1505–1512

Matsushita K, Ohno Y, Shinagawa E, Adachi O, Ameyama M (1982) Membrane-bound, electron transport-linked, D-glucose dehydrogenase of *Pseudomonas fluorescens*. Interaction of the purified enzyme with ubiquinone or phospholipid. Agric Biol Chem 46:1007–1011

Meiberg JBM, Spa MA (1983) Microbial production of gluconic acid and gluconates. Antonie van Leeuwenhoek 49:89–90

Mowshowitz S, Avigad G, Englard S (1974) 5-Keto-D-fructose: formation and utilization in the course of D-fructose assimilation by *Gluconobacter cerinus*. J Bacteriol 118:1051–1058

Neijssel OM, Tempest DW, Postma PW, Duine JA, Frank Jzn J (1983) Glucose metabolism by K^+-limited *Klebsiella aerogenes*: evidence for the involvement of a quinoprotein glucose dehydrogenase. FEMS Microbiol Lett 20:35–39

Olijve W (1978) Glucose metabolism in *Gluconobacter oxydans*. PhD Thesis, University of Groningen, The Netherlands

Olijve W, Kok JJ (1979a) Analysis of growth of *Gluconobacter oxydans* in glucose containing media. Arch Microbiol 121: 283–290

Olijve W, Kok JJ (1979b) An analysis of the growth of *Gluconobacter oxydans* in chemostat cultures. Arch Microbiol 121:291–297

Schie BJ van, Dijken JP van, Kuenen JG (1984) Non-coordinated synthesis of glucose dehydrogenase and its prosthetic group PQQ in *Acinetobacter* and *Pseudomonas* species. FEMS Microbiol Lett 24:133–138

Schie BJ van, Hellingwerf KJ, Dijken JP van, Elferink MGL, Dijl JM van, Kuenen JG, Konings WN (1985) Energy transduction by electron transfer via a pyrrolo-quinoline quinone-dependent glucose dehydrogenase in *Escherichia coli, Pseudomonas aeruginosa* and *Acinetobacter calcoaceticus* (var *lwoffi*). J Bacteriol 163:493–499

Schie BJ van, Mooy OH de, Linton JD, Dijken JP van, Kuenen JG (1987) PQQ-dependent production of gluconic acid by *Acinetobacter, Agrobacterium* and *Rhizobium* species. J Gen Microbiol 133:867–875

Shchelkunova SA, Voinova GN (1969) Influence of the conditions of culturing on the formation of the calcium salt of 5-keto-gluconic acid by acetic acid bacteria. Mikrobiologiya (Engl transl) 38:583–588

Shinagawa E, Matsushita K, Adachi O, Ameyama M (1983) Selective production of 5-keto-D-gluconate by *Gluconobacter* strains. J Ferment Technol 61:359–363

Shinagawa E, Matsushita K, Adachi O, Ameyama M (1984) D-Gluconate dehydrogenase, 2-keto-D-gluconate yielding, from *Gluconobacter dioxyacetonicus*. Purification and characterization. Agric Biol Chem 48:1517–1522

Stadler-Szöke A, Nyeste L, Holló J (1980) Studies on factors affecting gluconic acid and 5-keto-D-gluconic acid formation by *Acetobacter*. Acta Aliment 9:155–172

Stubbs JJ, Lockwood LB, Roe ET, Tabenken B, Ward GE (1940) Bacterial production of ketogluconic acids from glucose. Ind Eng Chem 32:1626–1631

Vishniac W, Santer M (1957) The thiobacilli. Bacteriol Rev 21: 195–213

Weenk G, Olijve W, Harder W (1984) Ketogluconate formation by *Gluconobacter* species. Appl Microbiol Biotechnol 20:400–405

Received September 25, 1987/Accepted November 30, 1987